Inflorescence Sampling Improves Effective Population Size of Grasses

R. C. Johnson,* V. L. Bradley, and M. A. Evans

ABSTRACT

Variation in seed production per plant leads to reductions in effective population size (N_e), which is a major factor promoting genetic drift of heterogenetic populations during seed collection and regeneration. The objectives of this study were (i) to compare N_e to the census population size (N_c) among different seed harvest methods; (ii) use inflorescence sampling to determine N_e/N_c for numerous heterogenetic grass species, and (iii) to predict the optimum number of inflorescences per plant to most efficiently increase N_e. Estimates of N_e/N_c from rubbing whole plants, cutting whole plants, and from sampling a constant two inflorescences per plant were made on *Festuca pratensis* Huds., *Lolium perenne* L., and *Pseudoroegneria spicata* (Pursh) Á. Löve accessions. Inflorescence sampling was also completed on four accessions of *Bromus inermis* Leyss., *Dactylis glomerata* L., *F. arundinacea* Schreb., *L. perenne*, *Pseudoroegneria spicata*, and *Phalaris aquatica* L. The mean N_e/N_c for the inflorescence method was 0.78, significantly higher then the 0.64 average for the cut or rub methods. For all species and entries, the slope of the curves describing N_e/N_c to inflorescence number was initially steep from 1 to 3 inflorescences and then leveled off asymptotically. Thus, most of the benefit occurs after sampling only a few inflorescences. The results show that sampling a constant number of inflorescences per plant promotes N_e and reduces the potential for genetic drift associated with collection and regeneration of heterogenetic grass populations.

The Western Regional Plant Introduction Station (WRPIS) maintains more than 17 000 forage and turf grass accessions. The majority of these are self-incompatible, wind-pollinated species with high levels of heterogeneity. Initial and periodic seed regeneration is needed to maintain this collection (Johnson et al., 2002). The potential for random genetic drift is a major concern in the relatively small populations associated with regeneration. The N_e rather than the N_c is the key parameter in genetic drift, and is defined as the size of an ideal population that would have the same amount of random genetic drift as the actual census population (Wright, 1931; Crow and Kimura, 1970). A major cause of reduced N_e is variation in fecundity or seed production per plant (Heywood, 1986). In some cases, mating can be controlled so that every individual in a population contributes two gametes to the next generation; N_e is then essentially doubled compared with N_c (Crow and Kimura, 1970). Other regeneration schemes to maximize N_e include various types of controlled polycrosses (Breese, 1989). When male and female gametes are uncontrolled and seeds are bulk harvested, variation in fecundity can substantially reduce N_e compared with N_c.

Johnson et al. (2002) found that variation in seeds per whole plant in grass populations caused a sharp decline in N_e. They also found that by sampling a constant two inflorescences per plant that N_e was increased almost 60% compared with whole-plant sampling. In that study, comparisons of N_e/N_c for different whole-plant sampling methods with inflorescence sampling were not made. Also lacking were N_e/N_c values derived from inflorescence sampling of numerous grass species, and information relating the number of inflorescences sampled to N_e/N_c. The objectives of this study are (i) to compare N_e/N_c among different harvest methods, (ii) to test inflorescence sampling among numerous grass species at the WRPIS, and (iii) to predict the optimum number of inflorescences to sample to most efficiently increase N_e.

MATERIALS AND METHODS

Calculation of N_e/N_c

The method for calculation of N_e/N_c used herein was developed by Heywood (1986) as outlined in Johnson et al. (2002). A similar approach by Robertson (1961) was discussed by Crow and Kimura (1970). Briefly, Heywood (1986) related variation in seed production per plant with the number of gametes individuals contribute to the population gamete pool. Variation in fecundity resulting from uneven contributions of gametes is a major factor reducing N_e and enhancing genetic drift. With Heywood's (1986) equation:

$$N_e/N_c = 1/[(1 + F)\ (\sigma^2/\mu^2) + 1], \text{ and}$$
$$= 1/[(1 + F)\ (\sigma_b^2/\mu^2 + \sigma_w^2/m\mu^2) + 1], \quad [1]$$

where F is the fixation index (inbreeding coefficient), and σ and μ are the standard deviations among plants and the mean family size, respectively. We have further partitioned σ^2/μ^2 into the between-plant (σ_b^2) and within-plant (σ_w^2) variance components in relation to m, the number of inflorescences per plant. When each parent contributes equally to the gamete pool, σ^2 is zero and $N_e = N_c$. It can also be seen that as m increases, the within-plant variance component will decrease and lead to a decrease in σ^2/μ^2. An estimate of σ^2/μ^2 is given by $s^2/z^2 - 1/z$, where s^2 is the sample variance in seed number among plants and z is the sample mean seed number per plant in a given population. Often, seed production is high enough that the correction term, $1/z$, has little effect.

Field Experiments Comparing Harvest Methods for N_e/N_c

Estimates of N_e/N_c were made based on seed number per plant from three accessions, one each from three perennial grass species maintained at the WRPIS. The three entries were *F. pratensis* (W6 17784), *L. perenne* L. (W6 9344), and *Pseudoroegneria spicata* (PI 236681). The same accessions were also used in estimates of N_e/N_c by Johnson et al. (2002). These species are outcrossing, wind pollinated, and self-incompatible, so a value of $F = 0$ was assumed for the calculations of N_e/N_c (Brown, 1979; Johnson, 1998; Johnson et al., 2002).

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Abbreviations: N_c, census population size; N_e, effective population size.

Plants of each entry were established at Central Ferry (46°40′13″ N and 117°45′8″ W) and Pullman (46°43′55″ N and 117°9′25″ W), WA, locations. The soil at Central Ferry is a fine-silty, mixed, mesic Natrixeroll and at Pullman a fine-silty, mixed, mesic, Pachic Ultic Haploxeroll. Central Ferry is located approximately 50 km west of Pullman in the Snake River Canyon at about 200 m in elevation, about 500 m lower than Pullman. Plants at Central Ferry were grown under irrigation as described previously (Johnson and Li, 1999). Associated with its lower elevation and more westerly location toward the Columbia Basin desert, temperatures at Central Ferry average about 3.5°C higher and precipitation about 200 mm lower per year than at Pullman.

Plants of each accession were established in a greenhouse and transplanted to the field in the second week of April 2000 at Central Ferry and the second week of May 2000 at Pullman as described by Johnson et al. (2002). Field plot cultivation and fertilization were as described by Johnson et al. (2002).

Four blocks at each location were split into plots of 30 plants representing each accession. The 30 plants per plot were spaced 0.5 m within rows and 1.8 m between rows. Accessions (entries) were isolated from other accessions of the same species by at least 50 m. As expected, there was minimal seed production during the establishment year. Plants were maintained during the spring and summer of 2000 and mowed to about a 20-cm height in the fall.

In the spring of 2001, the 30 plants in each plot were randomly spilt into three subplots of 10 plants representing three harvest methods. Thus, the design was a randomized complete block in a split-plot arrangement with four replications at two locations. The harvest methods were designated as cut, rub, and inflorescence sampling. For the cut method, each of the 10 plants in a given subplot was cut with a sickle and placed in a bag. This was done when the maximum number of seeds was judged to have reached physiological maturity. For the rub method, seed from each plant was rubbed into a pan and placed in a bag. Rubbing of plants was repeated to obtain seeds from inflorescences maturing at different times. The inflorescence method consisted of sampling two inflorescences from each plant judged to be physiologically mature. The inflorescences were kept separate for estimates of variation in seeds per inflorescence. All cut, rub, and inflorescence samples were harvested during the spring and summer of 2001.

For the cut and rub methods, harvested material from each plant was cleaned and seeds weighed to obtain yield per plant. Seed mass was determined by counting and weighing 100 seeds per plant. The number of seeds per inflorescence was calculated by the equation

$$\text{yield plant}^{-1} = \text{inflorescences plant}^{-1} \times \text{seeds inflorescence}^{-1} \times \text{weight seed}^{-1}.$$

For the inflorescence method, the two inflorescences sampled were cleaned, and the seeds were counted and weighed. The seeds from the two inflorescences represented the total seeds per plant sampled for the inflorescence method. For each method, the variance and mean seeds per plant were used to estimate N_e/N_c as described in Eq. [1]. Data were analyzed across locations with SAS GLM release 6.12 (SAS Institute, 1985), assuming fixed effects for location, entry, and method and random effects for blocks. Thus, inferences were made only for the Central Ferry and Pullman locations. The error terms used for testing location was the block within location mean square. The error term for testing entries and the entry × species interaction was the block × species within location mean square. For tests involving method and its interactions, the residual error term was used. Treatment differences were declared at $P < 0.05$ (SAS Institute, 1985). For the analysis of inflorescence number per plant, the inflorescence sample with a constant two per plant was not included since it was invariant and would otherwise skew the analysis. Similarly, seed weight per plant (yield) was analyzed for only cut and rub methods. Partial coefficients of determination (R^2) were calculated as the ratio of the sum of squares of a given treatment factor to the total sum of squares (Neter et al., 1996). This gave the proportion of variation explained by location, entry, method, and associated interactions. Pearson's correlation coefficient was also computed for development and seed production factors.

Survey of Species N_e/N_c by Inflorescence Sampling

In addition to the above experiment, a survey of N_e/N_c using the inflorescence sampling method was completed with species in WRPIS regeneration nurseries during 2001. Inflorescence samples from a total of six species represented by four accessions each were sampled at Central Ferry and Pullman. The species analyzed were *B. inermis*, *D. glomerata*, *F. arundinacea*, *L. perenne*, *Pseudoroegneria spicata*, and *Phalaris aquatica*. The species are all wind-pollinated and highly outcrossing (Fryxell, 1957), so F in Eq. [1] was assumed to be zero. For each accession, two inflorescences were sampled separately from each of 15 plants, cleaned, and total seeds counted. The standard deviation and mean seed number was determined, and Eq. [1] was used to estimate N_e/N_c.

Within each species at each location, four accessions were randomly selected. Thus, the accessions are random effects and represent the units of replication for this design. The experiment was a completely randomized design with a two-way treatment structure (location and species). The accession within species × locations term was the residual error and used for comparing differences among species. Estimates of N_e/N_c were made as described above for each plot. Data were analyzed across locations with SAS GLM release 6.12 (SAS Institute, 1985), assuming fixed effects for location and species with inferences confined to the Central Ferry and Pullman locations. Treatment differences were declared at $P < 0.05$ (SAS Institute, 1985). The R^2 values for location, species, and the location by species interaction were calculated as described above (Neter et al., 1996).

Modeling the N_e/N_c Response to Inflorescence Number

Estimates of the relationship between inflorescence number sampled per plant and N_e/N_c were made based on the estimated sample mean (z) and variance (s^2) when inflorescence number was $m = 2$, the number sampled in the studies described above. For the inflorescence sampling, a random sample of n plants was selected with a random sample of two inflorescences selected from each plant, resulting in a completely randomized design with $m = 2$. The ANOVA model for this design partitions the variance into between-plant variation and within-plant components.

Let Y_{ij} represent the number of seeds on the jth inflorescence ($j = 1, 2, \cdots, m$) of the ith plant ($i = 1, 2, \cdots, n$). The estimator of the mean number of seeds per inflorescence for the ith plant is

$$\overline{Y}_{i\cdot} = \frac{\sum_{j=1}^{m} Y_{ij}}{m}$$

and the mean number of seeds per inflorescence across all plants is

$$\overline{Y}_{\cdot\cdot} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m} Y_{ij}}{n \cdot m}.$$

On the basis of these two quantities, the formula for the mean square between plants is

$$MS_{between} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m}(\overline{Y}_{i.} - \overline{Y}_{..})^2}{n-1} = \frac{\sum_{i=1}^{n} m_i(\overline{Y}_{i.} - \overline{Y}_{..})^2}{n-1},$$

and the mean square within plants is

$$MS_{within} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m}(Y_{ij} - \overline{Y}_{i.})^2}{n(m-1)},$$

Under the completely randomized design, the expected value of the mean squares are $E(MS_{between}) = m\sigma_b^2 + \sigma_w^2$ and $E(MS_{within}) = \sigma_w^2$, where σ_b^2 represents the between-plant variance component and σ_w^2 represents the within-plant variance component. An estimator of σ_w^2 is the MS_{within} and an estimator of σ_b^2 is $(MS_{between} - MS_{within})/m$.

For the estimation of N_e/N_c, with sampling based on a completely randomized design, the variable of interest is the number of seeds sampled per plant, or

$$Y_{i.} = \sum_{j=1}^{m} Y_{ij}.$$

The expected value of $Y_{i.}$ is μ and the variance is $\sigma^2 = m^2\sigma_b^2 + m\sigma_w^2$. Thus, an estimator of μ is

$$z = \frac{\sum_{i=1}^{n} Y_{i.}}{n}$$

and an estimator of σ^2 is

$$s^2 = [m^2(MS_{between} - MS_{within})/m] + m \times MS_{within} = m \times MS_{between}.$$

Substituting these values into Eq. [1] provides an estimate of N_e/N_c.

Estimates of how N_e/N_c varies as $m^* = 1, 2, \cdots, M$, the desired inflorescence number, can be obtained. The mean number of seeds sampled per plant can be estimated by $z(m^*) = m^* \times z/m$ and the variance in the number of seeds sampled per plant can be estimated by

$$s^2(m^*) = m^{*2} \times (MS_{between} - MS_{within})/m + m^* \times MS_{within}.$$

Here, z, $MS_{between}$ and MS_{within} were computed from the original data with $m = 2$. Substituting the values of $z(m^*)$ and $s^2(m^*)$ into Eq. [1] provides an estimate of N_e/N_c for $m^* = 1, 2, \cdots, M$. Estimates of $s^2(m^*)$ and $z^2(m^*)$ were made with inflorescence samples collected in the harvest methods study and the species survey study as outlined above.

RESULTS AND DISCUSSION

Comparing Harvest Methods for N_e/N_c

For seeds per plant, all treatment factors and interactions were significant, with the method effect showing the highest fraction of variation (Table 1). This was expected, given that the inflorescence sample, with a constant two, had much fewer seeds per plant then the cut and rub samples, which represented all inflorescences per plant. The entry effect had the most variation for seeds per inflorescence, but all interactions were significant (Table 1). For inflorescences per plant, with data from only the cut and rub methods, the only significant treatment factor was entry, accounting for nearly 83% of the variation. Much of this was because the *L. perenne* had 544 inflorescences per plant compared with only 133 for the *F. pratensis* entry and 135 for the *Pseudoroegneria spicata* entry. Nearly 95% of the variation for seed mass was associated with entry effects (Table 1). All entries differed in seed mass: *F. pratensis* averaged 1.97 mg, *L. perenne* 1.51 mg, and *Pseudoroegneria spicata* 4.60 mg. Since each entry represented a different species, it was expected that large entry effects would be observed for different yield components, and they were.

For N_e/N_c, the location effect, the method effect, and the location × entry interaction were significant (Table 1). The Pullman location had a higher overall N_e/N_c at 0.75 than the Central Ferry location at 0.63. The interaction with entry resulted because N_e/N_c did not increase in Pullman for *Pseudoroegneria spicata*, averaging 0.65, as it did for *L. perenne*, which increased from 0.59 at Central Ferry to 0.84 at Pullman.

For *F. pratensis* and *L. perenne*, the Pullman environment was more favorable for seed production then the Central Ferry environment, as seen by the sharp increase in seeds per plant in the cut and rub treatments (Table 2). This was associated with increased seeds per inflorescence at Pullman. For *Pseudoroegneria spicata*, however, there was no difference detected between locations (Table 2). Inflorescences per plant were generally unaffected by loca-

Table 1. Summary of the mean, CV, R^2 values (percentage variation explained), and statistical significance resulting from ANOVAs for attributes associated with seed production and the ratio of effective to census population size (N_e/N_c). The locations were Pullman and Central Ferry, WA, in 2001. Entries were *Festuca pratensis* (W6 17784), *Lolium perenne* (W6 9344), and *Pseudoroegneria spicata* (PI 236681). Harvest methods were hand-cutting all plants, rubbing all plants, or sampling two inflorescences per plant.

Attributes	Mean	CV	Location (L)	Entry (E)	L × E	Method (M)	E × M	L × M	E × L × M
						R^2			
Seeds plant^{-1}	5587	30.9	12.5**	20.7**	6.4**	30.1**	10.0**	4.9**	7.5**
Seeds inflorescence^{-1}	59.9	21.8	21.8*	37.2**	18.1**	10.5**	4.7**	2.2**	1.2**
Inflorescence plant^{-1}‡	271	34.3	2.1ns†	82.8**	1.3ns	<0.1ns	0.2ns	<0.1ns	0.5ns
Seed mass, mg	2.69	10.7	0.5**	94.8**	0.3ns	<0.1ns	0.7*	0.1ns	0.2
Plant yield, g‡	16.8	21.0	36.4**	22.9**	17.8**	2.1ns	<0.1ns	<0.1ns	4.0**
N_e/N_c§	0.69	17.8	14.7**	4.8ns	10.3*	16.8**	3.6ns	<0.1ns	3.7ns

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
† ns, not significant at $P < 0.05$.
‡ Analysis presented for the cut and rub methods only since inflorescences per plant for the inflorescence sample was always two.
§ Calculated as $N_e/N_c = 1/[(1 + F)(\sigma^2/\mu^2) + 1]$, where N_e and N_c are the effective and census population sizes, respectively; F is the fixation index (assumed to be zero); and σ^2/μ^2 was estimated as $s_z^2/z^2 - 1/z$ for seeds per plant.

Table 2. Means for seeds per plant sample, seeds per inflorescence, inflorescences per plant, and seed mass for different locations, entries, and methods of seed harvest. The entries were *Festuca pratensis* (W6 17784), *Lolium perenne* (W6 9344), and *Pseudoroegneria spicata* (PI 236681), and data were collected in 2001. Harvest methods were hand-cutting all plants, rubbing all plants, or sampling two inflorescences per plant.

Entry	Location	Method	Seeds plant^{-1}	Seeds inflorescence^{-1}	Inflorescence plant^{-1}	Seed mass
						mg
F. pratensis						
	Central Ferry, WA	cut	5 498c*	29.1d	178.5a	1.81b
		rub	4 485c	27.8d	155.9a	1.89b
		inflorescence	163d	81.5c	(2)†	1.64b
	Pullman, WA	cut	10 003b	151.1b	76.0a	2.30a
		rub	17 289a	149.2b	123.8a	2.01a
		inflorescence	553d	276.7a	(2)	2.18a
L. perenne						
	Central Ferry, WA	cut	4 903c	9.1d	598.4a	1.49ab
		rub	8 949b	14.6d	606.6a	1.47ab
		inflorescence	53d	26.7cd	(2)	1.29b
	Pullman, WA	cut	21 545a	42.3bc	515.4ab	1.57ab
		rub	19 080a	43.9b	454.6b	1.76a
		inflorescence	239d	119.7a	(2)	1.50ab
P. spicata						
	Central Ferry, WA	cut	1 233a	10.4a	119.6a	4.55b
		rub	2 526a	17.3a	149.5a	4.48b
		inflorescence	46a	24.6a	(2)	4.75ab
	Pullman, WA	cut	1 872a	13.1a	141.6a	4.47b
		rub	2 078a	15.9a	130.8a	4.39b
		inflorescence	50a	24.8a	(2)	4.97a

*** Within an entry, means within a column followed by a different letter are significantly different at $P < 0.05$ with the LSD.**
† The (2) refers to the two inflorescences sampled per plant.

tion except for a lower value for *L. perenne* observed in the rub method at Pullman than at Central Ferry (Table 2). The general trend toward increased seed mass at Pullman compared with Central Ferry was most pronounced for *F. pratensis* (Table 2).

Johnson et al. (2002), working with the same entries as in this study, found that rubbed *F. pratensis, L. perenne*, and *Pseudoroegneria spicata* had N_e/N_c values of 0.56, 0.39, and 0.59, respectively, compared with 0.74, 0.65, and 0.60 in the current data. So, environment played an important role in the N_e/N_c value for different accessions, as might be expected given that N_e/N_c is based on seed production factors that often vary with environmental conditions.

For N_e/N_c, none of the interactions with method were significant, showing that method means were consistent across location and entry. The significant method effect for N_e/N_c showed inflorescence sampling improved N_e and confirmed the overall advantage of harvesting a constant number of inflorescences per plant suggested by previous work (Johnson et al., 2002). The mean N_e/N_c for the cut and rub methods were not significantly different (0.63 and 0.66, respectively). The inflorescence sample, however, with an N_e/N_c of 0.78, was higher than either the cut or rub harvest methods. In other words, if seeds from 100 plants were harvested and bulked, variation in seed production per plant would lower N_e to about 65 plants. If two inflorescences per plant were harvested and bulked, N_e would be lowered to just 78 plants. For this study, that was a 20% increase. In the Johnson et al. (2002) study with the same entries, N_e/N_c values averaged 0.81 for the inflorescence samples and only 0.51 for the whole-plant samples. This represents a 59% average increase in N_e/N_c attributed to inflorescence sampling. The larger effect of increased N_e/N_c for Johnson et al. (2002) then in the current study was mostly attributed to a lower N_e/N_c for whole plant than in the current study rather then substantial differences in N_e/N_c for inflorescence samples. Thus, seeds per whole plants appeared to vary with environment more that for inflorescence sampling.

The increase in N_e/N_c with inflorescence sampling can be attributed to two factors. First, by taking a constant number of inflorescences per plant, the variation associated with inflorescences per plant is completely eliminated as a factor contributing to the variation in seeds per plant. This is an important reason why variability in whole-plant seed production would be expected to be greater than for inflorescence sampling. Second, as more and more inflorescences are sampled per plant, the variation in seed number within plants is reduced, and this in turn reduces the total between-plant variance as shown in Eq. [1]. Thus, sampling a constant number of inflorescences per plant should maintain a higher N_e and reduce the potential for genetic drift compared with whole-plant sampling.

Survey of Species N_e/N_c by Inflorescence Sampling

The survey of six species at Central Ferry and Pullman by inflorescence sampling showed significant location, species, and location × species effects for seeds per plant, but no difference for N_e/N_c (Table 3). Results in Table 1 showed that location and entry may interact for N_e/N_c; that is, entry N_e/N_c may vary depending on environment. The results in Table 3 show, however, that variation of entries within species for N_e/N_c was large enough that differences among species were not observed. If they had been observed, it would mean that the optimal number of inflorescences sampled may vary with species, suggesting a different inflorescence number per plant would be needed for a given species to maintain a given N_e/N_c. Since this

Table 3. Summary of the mean, CV, R^2 values (percentage variation explained), and statistical significance resulting from ANOVAs for seeds sampled per plant and the ratio of effective to census population size (N_e/N_c). Samples of two inflorescences per plant were obtained from six grass species and four accessions within each species. The locations were Central Ferry and Pullman, WA, in 2001.

	Mean	CV	Location (L)	Species (S)†	L × S
			R^2		
Seeds per plant	341.8	31.9	8.5**	75.7**	5.0*
N_e/N_c	0.78	12.9	<0.1ns‡	12.3ns	16.4ns

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
† Species sampled were *Bromus inermis*, *Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne*, *Pseudoroegneria spicata*, and *Phalaris aquatica*.
‡ ns, not significant at $P < 0.05$.

was not the case, a single inflorescence sampling protocol for constant inflorescence number appeared most practical and efficient.

Modeling the N_e/N_c Response to Inflorescence Number

Increasing inflorescence number sampled per plant generally increased N_e/N_c as expected (Fig. 1). Although the effect varied in the amount of gain, the basic shape of the curve was the same for all entries at both locations. There was an initial steep response as inflorescence number increased from 1 to about 3, followed by an asymptotic leveling off (Fig. 1). It was also apparent that N_e/N_c varied depending on the location and entry. For example, the location × entry interaction for N_e/N_c (Table 1) was observed in the generally lower values for *Pseudoroegneria spicata* at Pullman compared with other entries at Pullman (Fig. 1). In addition, the initial slope of the curve to increasing inflorescence number was greater at Central Ferry than at Pullman. The reasons for differing initial slopes and maximal responses is related to how the variation between plants is partitioned. When the within-plant variance σ_w^2 is large compared with the between component σ_b^2, then sampling additional inflorescences m will give a larger, more positive response to N_e/N_c. If the within-plant variation is initially low compared with the between-plant component, then the response to increased inflorescences per plant is more muted. For example, the values of N_e/N_c for *Pseudoroegneria spicata* at Pullman were about equal to those of *F. pratensis* at Central Ferry when inflorescence number was two to four (Fig. 1). Yet, the initial slope and the values at higher inflorescence numbers were lower for *Pseudoroegneria spicata* at Pullman than for *F. pratensis* at Central Ferry. This was because the between-plant variance component for *Pseudoroegneria spicata* at Pullman was more than five times larger than the within-variance estimate, but the between-plant variance estimate for *F. pratensis* at Central Ferry was actually less than the within-variance estimate. This higher relative within-variance component for *F. pratensis* at Central Ferry allowed the more pronounced increase in N_e/N_c with increasing inflorescence number m (Fig. 1). As seen in Eq. [1], increasing m works to reduce the contribution of the within-variance component, which reduces the overall variance in seeds per plant. This effect was also seen between the locations: a higher relative within-plant variance lead to the more pronounced increase in N_e/N_c with m at Central Ferry compared with Pullman (Fig. 1).

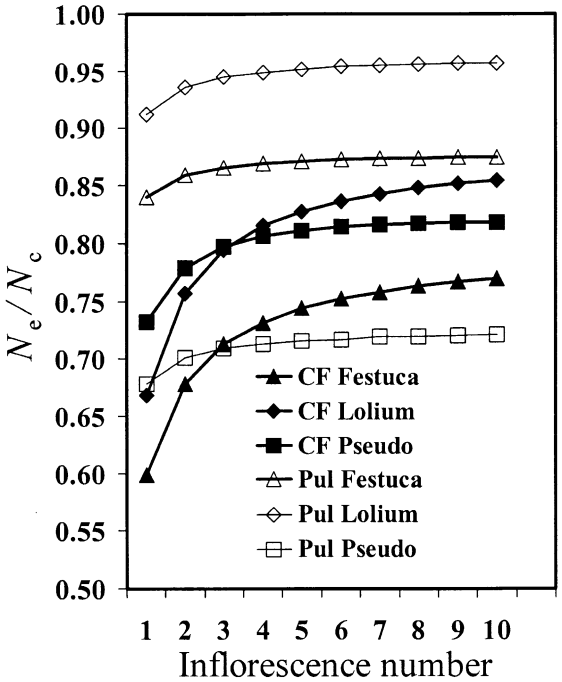

Fig. 1. Relationship between the ratio of effective to census population size (N_e/N_c) and increasing inflorescences sampled per plant for *Festuca pratensis*, *Lolium perenne*, and *Pseudoroegneria spicata* grown at Central Ferry (CF) and Pullman (Pul), WA, in 2001.

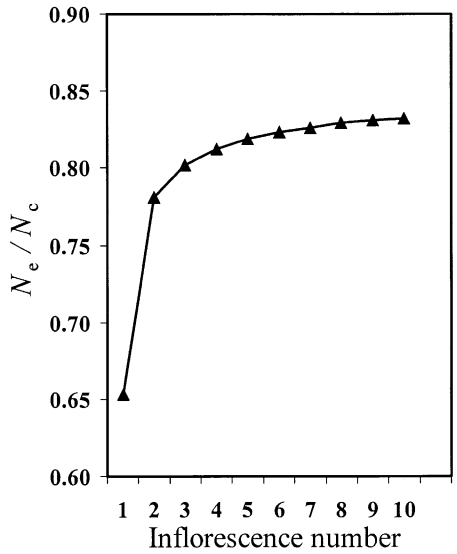

Fig. 2. Relationship between the ratio of effective to census population size (N_e/N_c) and increasing inflorescences sampled per plant averaged for four accessions each of *Bromus inermis*, *Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne*, *Pseudoroegneria spicata*, and *Phalaris aquatica* grown at Central Ferry and Pullman, WA, in 2001.

The relationship between N_e/N_c and inflorescence number for the survey of six species showed the same basic curve shape (Fig. 2) observed for the entries in Fig. 1. The initial response was quite strong as N_e/N_c increased from 0.65 to 0.77 as inflorescence number increase from one to two. As before, the curve started to level off after about three inflorescences. The steep initial slope resulted from a favorable balance of the within and between variance components as was observed for Central Ferry entries in Fig. 1.

Regardless of the difference in gain associated with increasing the inflorescence number sampled per plant, most of the effect is seen before three to five inflorescences. Thus, most of the benefits are realized after only a few inflorescences are sampled. The details of slope and maximal effects for inflorescence sampling to improve N_e/N_c will vary with growing environment and with individual entries, but the basic dynamics and shape of the relationship between inflorescence number and N_e/N_c should remain the same regardless of accession, species, and environment. Therefore, constant inflorescence sampling should be considered in other species, during field collection of germplasm, and in selection programs to help maximize N_e and the diversity of heterogenetic populations. This study was undertaken to advance and clarify sampling procedures used for regeneration of grasses to maintain as high N_e as possible with minimal inputs. We considered the maternal effects of sampling seeds but not the paternal pollen effects. Certainly methods that control male and female gametes will result in equal or even greater N_e than N_c (Breese, 1989). However, the resources to do this are often limiting, especially for large germplasm collections with large regeneration programs (Johnson et al., 2002). In these cases, such as at the WRPIS, inflorescence sampling is recommended as a cost-effective way to improve N_e.

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